Goals of this Study

1) To determine whether the coastal migratory stock is supplied with striped bass representative of distinct Mendelian populations. Do the three major spawning systems support significantly different gene pools, and therefore constitute different populations? Protein electrophoresis studies conducted in the past have failed to answer this question. (see pages 7-8)

2) Can restriction endonuclease analysis of mtDNA be used as an effective management tool to discrimate among striped bass of mixed ancestry in the coastal fishery? Will a composite mtDNA restriction genotype reveal fixed differences among the three major spawning systems, and therefore permit unequivocal identification of ancestry of individual striped bass? (see pages 8-12)

3) Does the Chesapeake Bay contain a single homogeneous population or do several different populations of striped bass inhabit the bay?(see pages 5 & 12)

4) Are there population subdivisions in the Hudson system? (see page 5)

5) Do spawning populations exhibit temporal stability in mtDNA genotype frequencies from year to year? (see page 5)

6) What is the extent of mtDNA sequence divergence among striped bass representative of the major breeding systems? (see page 8)

7) Does the mode and rate of mtDNA evolution in the fishes reflect that observed in the mammalian comparisons conducted to date? (see page 12)

8) Does the Connecticut River support a distinct spawning stock of striped bass or is there an annual influx of striped bass from other systems? (see page 5)

Protocol

Collections

Table 1 lists the dates and sites of striped bass collections. Adult striped

bass were collected at breeding time in the major spawning rivers. This was done to insure their being native to the rivers in which they were collected. An exception was the sample collected at Troy, New York during the month of July. Young-of-the-year and yearling striped bass obtained at various sites in the Hudson were assumed of Hudson ancestry due to the inability of southern stocks to undergo oceanic migrations till a later age (Kohlenstein, 1981). All fish were transported to our laboratory alive and maintained in holding tanks until processing. It should be noted that virtually all Chesapeake striped bass were male and primarily from a single year class.

mtDNA Purification and Characterization

mtDNA was obtained from freshly sacrificed striped bass. The details of the procedure are outlined in Wirgin and Grossfield (manuscript submitted). Briefly, the mtDNA was purified via multiple rounds of CsCl density gradient ultracentrifugation. Following digestion, DNA fragments were labelled with ^{32}P and visualized by autoradiography. Fragments obtained from most digests were aliquoted and separated on both agarose and polyacrylamide gels to visualize and score a range of fragments of widely different molecular sizes. Molecular sizes of all striped bass mtDNA fragments were determined by comparison to the appropriate DNA standards.

In total, 12 restriction enzymes were utililzed in characterizing the mtDNA genotypes of these fish. Not all enzymes were used in screening all striped bass. However, a minimum of 6 restriction digests were performed on each fish.

Mapping Procedures

Relative map sites on the striped bass mtDNA molecule for different restriction enzymes were determined by performing a series of double digests. In most cases single digests were followed by a round of alcohol precipitation to remove undesired buffer constituents before commencing second digests.

Results

General

Table 2A-C depicts the mean size and number of striped bass mtDNA fragments obtained for the various restriction enzymes used in this study.

Our strategy was to use several four and five base cutters because by producing multiple fragments, they would enable us to screen a sizeable proportion of the mitochondrial genome with a minimal number of restriction enzymes. In total, 155 fragments were produced, representing 730 base pairs. If we assume a mean striped bass mtDNA genomic size of 17820 base pairs (Wirgin and Grossfield, manuscript submitted), this constitutes 4.1 % of the entire striped bass mtDNA nucleotide sequence. Four-base-cutters produced a mean of 30 fragments, five-cutters 21 fragments, and six cutters 5 fragments.

Base Substitutions

Despite the use of several four and five base cutters, base substitutions were extremely rare among the population comparisons conducted. None of the six base cutters revealed any base sequence divergence. The five base cutters Ava II, and Hin FI also failed to reveal any nucleotide sequence divergence despite generating a large number of mtDNA fragments (42). The four base cutter Rsa I, revealed the greatest amount of base sequence heterogeneity among the individuals sampled. Four rare genotypes were observed, each in a single fish. All other striped bass displayed the common Rsa I genotype. Three of these unique Rsa I genotypes are exclusively found in the Rappahannock River while the fourth genotype was observed in a single fish from the Roanoke River. Taq I also generated a rare restriction genotype observed in only one fish from the Roanoke River. These five rare genotypes uniquely identify these individuals. It is interesting to note that all five rare genotypes are found in populations at the southern extreme of the range of the coastal migratory stock. Striped bass apparently harbor the lowest level of mtDNA sequence variation for any animal species surveyed in depth to date ($p=0.004$).

Length Polymorphisms

Macro size variation

Five different lengths of the mtDNA molecule were observed in the populations surveyed. Each of these lengths differed by approximately 100 base pairs from the next largest size. Therefore, the largest genotype was approximately 400 base pairs larger than the smallest.

All restriction enzymes revealed this length polymorphism with the exception of the six-base-cutter Xba I. Table 2 lists the fragment number

which displayed this molecular length variation for each enzyme. The difference in molecular size (kb) among the polymorphic fragments generated for all single enzyme digests was consistent for almost all enzymes used. In all cases this molecular length variation was seen in a single mtDNA fragment for each restriction enzyme used. With the exception of the four-cutter Mbo I, all alternate sizes of the polymorphic fragment were clearly seen between adjacent non-polymorphic fragments. For Mbo I, length variants appeared as doublets or comigrating bands. For the different length genotypes generated by Mbo I, therefore, different fragments appeared as doublets. This observation was confirmed via densitometry.

The fact that all length polymorphisms are recognizeable as single fragments suggests that the additions or deletions of mtDNA responsible for this macro-size variation are all confined to a single region of the mtDNA molecule. All of these macro-size variants were visualized on agarose gels. The different classes of size variants were most easily scored and molecular size determinations were most accurate when using four-or-five-base cutters. When using some six-cutters such as Pvu II, the polymorphic fragment is quite large(10,000 kb), making it difficult to accurately size the variants.

Three of the length genotypes were common among the populations surveyed, while two were rare. The frequencies of the five genotypes in the various populations are presented in Table 3. It should be noted that all populations displayed intrapopulation mtDNA size heterogeneity. No single length genotype was fixed any population. However, the frequencies of these length variants did differ signficantly among the individual rivers and overall systems surveyed. Table 4 provides a matrix of Chi Square values for all possible site comparisons.

Systems Comparisons

On the overall systems level, it may be seen that when comparing the **Chesapeake** total to the **Hudson** total, highly significant differences are observed. In addition the **Chesapeake** total also differs significantly from that observed in the **Roanoke**. However, no significant differences are observed when comparing **Hudson** to **Roanoke** totals suggesting a closer relatedness between these two systems.

Subsystem Comparisons

If we divide the Chesapeake system into two major components; **upper bay** (C & D Canal and Upper Bay-Aberdeen) or (C & D Canal, Upper Bay-Aberdeen, and Choptank River) and **lower bay** (James River and Rappahannock River), we find that both of these Chesapeake subsystems considered independently differ significantly from **Hudson** totals. However, it should be noted that the magnitude of this difference is far greater when comparing **upper bay** to **Hudson** totals. This observation suggests a closer relatedness between **Hudson** and **lower bay** stocks.

Surprisingly, when comparing **upper** and **lower** Chesapeake Bay frequences, no significant differences were observed. Similarily, no signicant differences were found when comparing **upper** and **lower** Chesapeake Bay frequencies independently to that observed in the **Roanoke** total.

Intrasystems Comparisons

No significant differences were observed when comparing the various tributaries of the Chesapeake Bay. Similarly, the Hudson stock proved homogeneous regarding this length characterization.

In addition, in those cases where sampling of a single system occurred in successive years, no significant differences in genotypic frequencies were observed

Connecticut River Derivation

mtDNA length frequencies in the Connecticut River were shown to differ highly significantly from those reported for the Chesapeake total precluding a recent Chesapeake origin for Connecticut River striped bass. With the exception of the James and Rappahannock Rivers in the lower bay, all other single sites listed for the Chesapeake showed highly significant differences in mtDNA length frequencies when compared to the Connecticut River. However, both the Hudson and Roanoke apparently share common gene pools with those observed in the Connecticut. It should also be noted, however, that both the Hudson and Connecticut rivers share a common rare length genotype which would suggest exchange between these two systems. Due to an absence of a unique Connecticut River length

genotype; sharing of a rare genotype, and similar major length genotypic frequencies, an exchange between the Hudson and Connecticut is postulated.

Micro size variation

One four-base cutter, Taq I, revealed micro size variation in two small fragments visualized on 6% acrylamide gels. Although both of these length genotypes were quite rare, they do prove useful in stock discrimation. Ten striped bass, of upper or mid-Chesapeake origin, displayed a unique Fragment #17 for this enzyme. Table 5 depicts the distribution of these Taq I fragments among Chesapeake sites. It should be noted that these microvariants were not found in either lower Chesapeake tributary; the James or Rappahannock rivers. Most importantly, these rare micro genotypes were also absent from the Hudson and Roanoke systems.

This rare fragment was 16 bp smaller than the common fragment reported to be 452 bp. Those striped bass exhibiting these unique micro size variants represented all three different major length genotypes. Fortuitously, one of these Chesapeake striped bass possessed the largest major length genotype (1), common to Hudson, but only observed in two Chesapeake samples. Its possession of this rare, but exclusively Chesapeake micro size variant, allowed for its discrimation from all Hudson samples.

In addition, one fish of Chesapeake ancestry also exhibited length variants in two other smaller fragments of 204 bp and 196 bp. These small length variants differed by 5 bp and 1 bp respectively from the common genotypes for these fragments. All of these micro variants were unique to the Chesapeake system and therefore allowed for unequivocal identification of ancestry of their carriers.

Heteroplasmy

Heteroplasmy refers to the expression of more than one mtDNA genotype in a single individual. The additional forms of the mtDNA molecule must be in sufficient copy number to allow for their detection. It is generally agreed that the minority form of the molecule must be present at a level

approximately 2-5 % of the majority form to achieve the threshold of detectability via current technqiues of DNA visualization (Avise and Lansman, I983). Assuming that there is absolutely no paternal inheritance to the developing embryo, heteroplasmy may result from one of two possible events. If a mutation has occurred during development in a somatic cell line, or if the prefertilized egg from which the individual fish developed was not fixed for a single form of the mtDNA, multiple forms of the mtDNA molecule would be observable.

We have observed a substantial level of heteroplasmy for the major length variants in our study (10%). These individuals, in addition to exhibiting the normal complement of fragments for any given enzyme digest, also presented one extra DNA band. This extra or heteroplasmic band was always adjacent to the DNA fragment which revealed length variation among the populations surveyed. In all cases the molecular size of this extra heteroplasmic band corresponded to that of other length variants observed among striped bass surveyed. Densitometric analysis of heteroplasmic bands revealed absorption values ranging from 5 % to approximately 50 % of adjacent non-heteroplasmic DNA fragments. Approximately 10% of striped bass surveyed displayed this form of heteroplasmy. This observation of heteroplasmy was not restricted to a single spawning system. All three major spawning systems contained striped bass displaying this phenomenon.

In all cases samples which we called heteroplasmic displayed this phenomenon as expected for all restriction enzymes used, with the exception of Xba I and Mbo I. Not surprisingly, all heteroplasmic fish exhibited extra fragments which corresponded to length genotypes found in other striped bass sampled. In addition, two samples showed more than one heteroplasmic genotype.

We have not used heteroplasmy as a tool in stock discrimination, however, there are frequency differences between populations. It might have potential in this regard, however, in the absence, of molecular data detailing the transmission genetics of the heteroplasmic state within the cellular population of individuals, we have no conception of the duration of time that this condition is maintained within individuals or between generations.

Conclusions

Stock Identification

Mendelian populations, and therefore unit stocks are commonly identified by significant differences in genotypic frequences among sites sampled. Given sufficient time since their isolation, individual stocks will accumulate significant differences in their gene pools as a result of random factors or differential selective pressures. If hypothesized distinct stocks do not exhibit substantial differences in genotypic frequences, the most plausible explanation is insufficient time since reproductive isolation.

Despite a wealth of protein studies addressing the problem of striped bass stock discrimination, to date none of these workers have demonstrated the existence of discrete unit stocks contributing to the coastal migratory stock (Otto, 1976; Grove and Powers, 1976; Wirgin and Grossfield, unpublished data). Morgan et al. (1973) claimed to be able to differentiate distinct populations among the various tributaries of the upper Chesapeake Bay by polyacrylamide gel electrophoresis of several protein products. However, subsequent work by Sidell et al. (1980) was unable to duplicate Morgan's results. A lack of detectable protein variation has precluded the demonstration of unique unit stocks. Protein monomorphism in striped bass is not soley restricted to the Atlantic coastal migratory stock. It is also observed among southeast (R. Wattendorf, pers. comm.) and Pacific populations of this species (Otto, 1976; Wirgin and Grossfield, unpublished data).

Based on isoelectric focusing of eye lens proteins, Saila et al. (1983) claim to be able to differentiate between representatives of these spawning systems. However, these workers were unable to find structural differences in these proteins among individuals surveyed. They did, however, observe significant differences in the concentration or expression of these protein products. The genetic basis for these protein concentration differences is not clearly understood. While these observed concentration differences may reflect frequency differences at underlying regulatory loci, an equally plausible explanation may be differential environmental or developmental cues impacting monomorphic regulatory loci. We have also examined eye lens proteins via isoelectric focusing in striped bass populations. No structural protein differences were detected.

Observed concentration differences were correlated with age and eye lens weight of the individual fish (Ralph, Wirgin, and Grossfield, unpub data).

Signficant differences in mtDNA major length genotypes have permitted us to differentiate between Hudson and Chesapeake stocks of striped bass. In addition, significant mtDNA genotypic differences are reported for Chesapeake versus Roanoke populations. This is the first demonstration of significant genetic differences between the heretofore hypothesized Mendelian populations.

However, in comparing Roanoke to Hudson samples we did not observe significant differences. Even given a larger sample size it is unlikely that this technique will reveal significant differences between the Hudson and Roanoke systems.. This similarity between Hudson and Roanoke samples is not totally unexpected. Earlier meristic studies by Raney and DeSylva (1954) suggested a genetic affinity between Hudson and Roanoke stocks.

mtDNA as an Innate Genetic Tag to Uniquely Identify Individuals.
Base Substitutions

One pivotal question concerns the utility of this approach in identifying individuals of unknown ancestry in the coastal fishery. The rarity of detected base substitutions limits the applicability of mtDNA sequence diversity as an effective tool in stock discrimation. Rsa I and Taq I permit the detection of 3 unique variants in the Rappahannock River (21%) and 2 unique variants in the Roanoke River (9%) respectively. Obviously, the striped bass with these base substitutions may be uniquely assigned as to river of ancestry. The Hudson River did not support any striped bass with unique base substitutions.

Macro Size Variants

Until very recently, almost all mtDNA vertebrate population surveys estimating genetic distance recorded substantial levels of base substitutions (Brown, 1985). In addition, intraspecific vertebrate mitochondrial genomic size was thought to be quite stable with the exception of small additions and deletions (1-10 bp). However, a single isofemale strain of the invertebrate species; Drosophila mauritiana, did exhibit major polymorphism in mitochondrial genomic size. Two variants differing in size by approximately 500 bp were reported (Solignac et al.

1984).

This contrasts with several recent studies which have reported considerable variability in the overall length of the mtDNA molecule in both invertebrate and vertebrate species. Densmore et al. (1985) and Harrison et al. (1985) have reported multiple size classes of mtDNA in parthenogenetic lizards (37) and crickets (3) respectively. While the crickets exhibited moderate levels of recognition site gain or loss, the lizards revealed unexpectedly low levels of base substitution accompanying these length polymorphisms ($p= 0.006$). It should also be noted that two distinct models of length polymorphism were detected in the lizard work; continuous and discrete variation. Two classes of discrete variants were observed differing in molecular size by 35 bp. Twenty nine continous size variants in a sample size of 92 individuals were reported with a maximum molecular size range of 370 bp. Our work with southeastern U.S. populations of striped bass has also disclosed extensive length polymorphism in the fish mitochondrial genome accompanying unexpectedly low levels of substitution (Wirgin and Grossfield, manuscript submitted). These discrete size classes of length variants differed from each other by approximately 100 bp.

Major mtDNA discrete length variation does offer some potential for stock discrimination. Three easily identifiable length variants were commonly encountered in the populations surveyed in addition to the two rare variants reported. The largest of these three genotypes (1) was observed in 32% of Hudson River striped bass. This A genotype was noted in two (3%) Chesapeake Bay striped bass: one from the Choptank River and one from the C & D Canal. Due to micro-variation in a Taq I fragment, however, the sample from the Choptank River was unequivocally distinguishable from all Hudson River striped bass. Therefore, all Hudson River striped bass possessing this genotype (32%) were distinguishable from all Chesapeake Bay striped bass except for the single specimen from the James River.

In addition, two (3%) of Hudson River striped bass displayed a rare large length genotype (1-A) also noted in a single Chesapeake sample from the Rappahannock River. However, the single Rappahannock striped bass bearing this genotype was uniquely identifiable due to its possession of

the rare aforementioned Rsa I base substitution not observed in the two Hudson River fish. By scoring all major length variants a total of 35% of Hudson River striped bass were uniquely identifiable from all Chesapeake samples except for the single specimen from the C & D Canal.

It should be noted that differences did exist in the frequencies of one of the other two major classes of length variants (2 & 3) between the Hudson and Chesapeake stocks. The second largest common genotype, (2); was observed in 57% of Chesapeake striped bass while 33% of Hudson striped bass displayed this genotype. The third common genotype, (3); was seen in about equal frequency between Chesapeake and Hudson samples; 33% and 32% respectively. If we limited our focus to the relative frequencies of these two genotypes (2 and 3) in the Upper Chesapeake Bay (Aberdeen and C & D Canal) to Hudson River, we found more dramatic differences than when considering the Chesapeake Bay in total. Frequencies of 71% and 25% were reported for the (2) and (3) genotypes respectively in the upper Chesapeake Bay. Although these frequencies differ considerably from those observed in the Hudson (33% (2), 32% (3)), these differences are not significant (Chi square value=2.211, df=1). Undoubtedly, larger sample sizes would point to significant differences between the Hudson and Upper Chesapeake.

In comparing Roanoke to Chesapeake samples, major length variation allows us to uniquely identify 17% of Roanoke striped bass. If we include rare Rsa I and Taq I base substitution variants detected exclusively in Roanoke collections, 26% of Roanoke striped bass may be uniquely identified.

When comparing Roanoke to Hudson River striped bass major length variation is of little ultility in stock discrimination.

Micro Size Variants

Small additions or deletions in the mitochondrial genome of humans (Aquadro and Greenberg, 1983 and Cann and Wilson, 1983) and domestic cattle (Hauswirth et al. 1984) have been documented. However, as reviewed by these workers, small addition and deletion events in humans were about one-fifth to one-half as frequent as base substitutions. In addition to major length variants, Densmore et al. (1985) also observed occasional minor length variants (1-9 bp) among lizards. This mode of

mtDNA evolution has not been reported in any fish mtDNA studies to date. Despite running high concentration polyacrylamide gels (6-8%) in our southeastern striped bass study, micro size variants were not detected (Wirgin and Grossfield, manuscript submitted).

In this current study, small addition/deletion events were detected at two Chesapeake spawning sites at moderate frequencies by the enzyme Taq I (see Table 5).. These micro size variants were viewed in three different Taq I fragments. One sample exhibited two Taq I fragments displaying micro variation. These variants differed by 1 to 16 base pairs from the common size reported for these fragments. Despite the use of several other four and five-base-cutters, no other restriction enzyme yielded fragments displaying these small differences. This is not unexpected, however, unless the sequence containing these small added/deleted DNA tracts is found in a small restriction fragment (10-300 bp), there is little chance of its being detected.

Micro size variants were only observed in the Chesapeake Bay. In total, 19% of Chesapeake striped bass exhibited unique Taq I micro variants. These Taq I genotypes were reported in 36% of Upper Bay (Aberdeen) and 60% of Choptank River samples. No micro variants were detected in the two lower Chesapeake Bay rivers sampled; the James and Rappahannock rivers. Surprisingly, we also failed to observe this genotype in any of our samples from the C & D Canal. In total, 48% of Choptank River and Upper Bay (Aberdeen) striped bass sampled displayed this genotype. In addition, both the Hudson and Roanoke Rivers failed to yield any micro variants. Thus, this micro genotype could be used to uniquely identify individual Chesapeake Taq I micro variants from all Hudson and Roanoke Rivers striped bass. Furthermore, Taq I micro variation could be used to uniquely identify population subdivisions in the Chesapeake.

Micro variants were observed in representatives of all three common classes of major length variants. A similar distribution of micro variants among major length variants in lizards was reported by Densmore et al. (1985). This suggests that the major and minor addition/deletion events occurred at a different sites in the mtDNA molecule. Scoring Taq I micro variants apparently can aid in distinguishing Upper Bay and Choptank River vs. lower Chesapeake Bay (James and Rappahannock) striped bass.

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Table 1
Sample Characterizations

Sample Site	Date	# Individuals	Sex	Size Range(SL)
Chesapeake Bay				
Choptank River	5/9/83	1	M(1),F(0)	271mm
Upper Bay, Aberdeen	5/10/83	10	M(10),F(0)	271-417mm
Choptank River	4/18/84	13	M(13),F(0)	245-319mm
Rapahannock River	4/25/84	14	M(14),F(0)	216-394mm
James River	4/26/84	11	M(11),F(0)	205-344mm
C & D Canal	5/8/84	14	M(14),F(0)	230-281mm
Roanoke River, North Carolina				
Weldon	6/1/83	8	M(8),F(0)	369-479mm
Weldon	5/17/84	15	M(15),F(0)	279-355mm
Hudson River				
Croton Bay	5/21/83	2	M(1),F(1)	490-639mm
Cornwall	6/5/83	4	M(4),F(0)	450-539mm
Seatrain, NJ	3/3/83	15	M(4),F(0)	114-212mm
Croton Bay	3/21/83	3	M(2),F(0)	320-330mm
G W Bridge	3/3/83	5	M(3),F(0)	205-269mm
125 St, NYC	6/20/83	4	M(0),F(0)	137-220mm
Danskammer	6/27/83	2	M(0),F(0)	207-210mm
ubby Hook	3/17/83	1	M(0),F(1)	306mm
Westway	12/17/83	15	M(6),F(1)	185-290mm
Cornwall	5/31/84	6	M(5),F(1)	392-552mm

Claverack	6/3/84	6	M(6),F(0)	429-525mm
Troy	7/15/84	9	M(0),F(0)	213-410mm
125 St, NYC	7/18/84	5	M(1),F(0)	162-255mm
Connecticut River	6/22/83	12	M(3),F(0)	205-255mm
Coastal Collection				
Montauk, NY	11/8/84	12	Unknown	501-850mm TL
Montauk, NY	11/11/84	12	Unknown	546-838mm FL

Table 2A- Mean Number and Size of mtDNA Fragments Produced by Four-Cutters

	Rsa I	**Taq I**	**Mbo I**
Fragment Number	Fragment Size	Fragment Size	Fragment Size
1	2816	Polymorphic	1861
2	Polymorphic	1702	1604
3	2136	1620	Polymorphic
4	1974	1381	1262
5	1204	1135	1163
6	1104	1085	1092
7	838	924	1023
8	703	840	925
9	498	806	865
10	459	776	809
11	431	735	735
12	405	687	690
13	366	627	642
14	346	597	526
15	330	509	410
16	317	492	381
17	303	452	349
18	254	325	336
19	222	257	321
20	206	213	298
21	172	204	266
22	103	196	213
23	96	187	148
24	71	118	115
25		115	98
26			90
27			70
28			63
29			47
30			32

Table 2B-Mean Number and Size of mtDNA Fragments Produced by Five-Cutters

	Ava II	Hin F I
Fragment Number	Fragment Size	Fragment Size
1	3715	Polymorphic
2	3126	1900
3	Polymorphic	1643
4	1926	1429
5	1651	1257
6	1184	1068
7	1146	868
8	693	625
9	653	603
10	515	560
11	383	533
12	222	509
13	208	495
14		483
15		460
16		428
17		391
18		361
19		348
20		323
21		222
22		195
23		167
24		117
25		111
26		97
27		92
28		59
29		41

Table 2C- Mean Number and Size of mtDNA Fragments Produced by Six-Cutters

	Ava I	Bgl II	Eco R I	Hind III
Fragment Number	Fragment Size	Fragment Size	Fragment Size	Fragment Size
1	3952	7737	6330	7971
2	3645	Polymorphic	5104	6033
3	2329	2792	2791	2296
4	2126	1947	Polymorphic	Polymorphic
5	1396			
6	1357			
7	1216			
8	Polymorphic			
9	417			
10	398			
11	261			

Table 2C- Mean Number and Size of mtDNA Fragments Produced by Six-Cutters

Pvu II	Sac I	Xba I
Fragment Size	Fragment Size	Fragment Size
Polymorphic	11277	5635
5608	Polymorphic	3535
1328	1276	3456
		3233
		1703

Table 3- Frequencies of Major Length Genotypes at Sampling Sites

Genotype	Lower Hudson	Cornwall	Claverack	Troy	Roanoke 1983	Roanoke 1984	James
1-A	2	0	0	0	0	0	0
1	11	3	1	4	3	1	0
2	9	7	2	2	3	4	6
3	11	2	3	3	3	9	6
4	0	0	0	0	0	0	0

Table 4. Chi Square Comparison of Frequencies of Major Length Genotypes between Sampling Sites

	Upper Bay, Aberdeen	Choptank River	C & D Canal
Upper Bay, Aberdeen			
Choptank River	2.57		
C & D Canal	1.797	1.059	
Rappahannock River	2.945	3.654	2.967
James River	2.121	2.547	1.857
Chesapeake Total	1.853	2.188	1.103
James & Rappahannock	3.08	5.103	3.613
Upper Bay and C & D	0.572	2.158	0.247
James, Rappahannock, Choptank	2.876	1.994	2.032
Upper Bay, C & D, Choptank	1.148	0.744	0.454
Roanoke 1983	5.435	3.494	3.207
Roanoke 1984	6.296	4.256	3.846
Roanoke Total	7.207	4.972	4.972
Troy, NY	7.769	5.727	5.574
Claverack, NY	4.053	1.837	1.664
Troy & Claverack	7.639	5.372	4.961
Cornwall, NY	2.909	2.595	1.773
Lower Hudson	9.787*	8.341	7.075
Hudson Total	8.556	9.014	5.803
Connecticut River, Mass	15.4****	12.175**	12.175**

Table 5
Frequencies of Taq I Microvariants at Chesapeake Spawning Sites

	Upper Bay	Choptank	C & D	Rappahannock	James
Common Genotype	7	4	10	12	10
Rare Genotype	4	6	0	0	0

Table 3- Frequencies of Major Length Genotypes at Sampling Sites

Rappahannock	C & D Canal	Choptank 1983	Choptank 1984	Upper Bay	Connecticut
1	0	0	0	0	1
0	1	0	1	0	2
6	9	1	7	8	0
6	4	0	4	2	9
0	0	0	1	0	0

Table 4. Chi Square Comparison of Frequencies of Major Length Genotypes between Sampling Sites

Rappahannock Riv	James River	Chesapeake	Total James & Rappahannoc	Upper Bay and C & D
0.962				
2.816	1.511			
0.234	0.493	2.811		
4.378	2.543	1.753	4.844	
1.445	1.097	0.745	1.585	3.411
5.779	2.913	1.741	6.337	0.733
5.453	4.667	11.52**	9.339	6.279
2.967	1.857	5.317	4.645	6.464
4.659	2.907	9.516*	6.243	7.949
7.521	6.708	18.3****	12.862**	9.861*
2.801	2.251	3.453	4.641	3.191
6.289	5.301	15.2****	10.133*	9.123
6.047	5.077	8.633	9.002	3.562
5.806	6.738	20.9****	10.909*	13.173**
5.759	5.937	20.3****	10.238*	11.974*
8.574	9.6*	16.3****	11.250**	16.8****

Table 4. Chi Square Comparison of Frequencies of Major Length Genotypes between Sampling Sites

James, Rappahannock, Choptank	Upper Bay, C & D, Choptank	Roanoke 1983	Roanoke 1984
3.261			
9.37	7.043		
3.645	7.009	3.207	
6.966	8.818	1.259	0.911
14.231***	11.521**	0.343	4.597
3.076	3.02	0.625	0.556
11.48**	10.486*	0.152	3.236
8.134	4.301	1.4	6.155
14.365***	16.538****	0.636	5.708
13.964***	15.977****	0.314	6.264
12.504**	18.548****	6.912	5.21

Table 4. Chi Square Comparison of Frequencies of Major Length Genotypes between Sampling Sites

Roanoke Total	Troy, NY	Claverack, NY	Troy & Claverack	Cornwall, NY
2.539				
0.019	1.25			
1.303	0.296	0.583		
4.248	2.748	2.225	3.022	
3.899	0.859	1.281	1.04	4.158
3.904	0.993	1.155	0.927	2.984
6.256	6.368	4.875	6.634	12.655**

Table 4. Chi Square Comparison of Frequencies of Major Length Genotypes between Sampling Sites

Lower Hudson	Hudson Total	Connecticut River
0.656		
7.625	7.625	